

Alternative Quality Control Parameters for Autobac Susceptibility Testing Disks: Use of Agar Diffusion Zone Size Results

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Received 28 October 1980/Accepted 27 January 1981

Persistent concerns about limitation in the assessment of the quality control of Autobac 1 (Pfizer Diagnostics Division, Groton, Conn.) led us to investigate an alternative method for monitoring the performance of Autobac susceptibility testing disks. Current methodology for quality control of the system provides data which are interpreted at the high end of a numerical scale; e.g., the control strain of *Escherichia coli* consistently exhibits a light-scattering index value of 1.00 for all antibiotics tested. This type of end-of-scale criterion may not detect individual antibiotic disk aberrations of individual clinical isolate susceptibilities. Disk diffusion testing allows a semiquantitative, continuous-scale determination and will detect test performance variations, unless the control strain is highly resistant. During a 6-month period daily quality control procedures for 10 Autobac antibiotics tested against control strains of *E. coli* and *Pseudomonas aeruginosa* were monitored, utilizing both Autobac 1 recommendations and disk diffusion susceptibility (Kirby-Bauer) methodology. Readings were carried out by one of six technologists. Zone sizes were within a range of ± 3 mm of a mean value of 99% of the tests with *E. coli* and within ± 3 mm for 98% of the tests with *Pseudomonas*. Reproducibility was excellent. The high reproducibility may be due to the disk manufacturing process, which provides rigorous disk preparation and acceptability standards, to strict laboratory storage procedure, and to our own careful assessment of disk cartridges before their use for clinical susceptibility testing. We recommend that each new cartridge be tested in this manner and that a similar procedure be considered for other automated procedures in which disks are used.

Persistent concerns about limitations of the assessment of quality control of Autobac 1 (Pfizer Diagnostics, Division, Groton, Conn.) led us to consider an alternative method for monitoring the performance of Autobac susceptibility testing disks. Current methodology for quality control of the system provides data which are interpreted at the high end of a numerical scale; e.g., the control strain of *Escherichia coli* consistently exhibits a light-scattering index value of 1.00 for all antibiotics tested (6). It was our concern that this type of end-of-scale criterion may not detect aberrations of individual clinical isolate susceptibilities. Disk diffusion testing allows a semiquantitative, continuous-scale determination and is likely to detect even modest test performance variations, unless the control strain is highly resistant (2). Therefore, we evaluated the Autobac quality control procedure by performing disk diffusion

testing in parallel, using the Autobac disks and Autobac control organisms.

MATERIALS AND METHODS

Antibiotic disks. Antimicrobial agents used in this investigation were compounds used for susceptibility testing by the Autobac procedure in our clinical microbiology laboratory. Table 1 lists all antimicrobial agents for which susceptibility testing was carried out during the period of this evaluation. It should be recognized that these compounds were used in different combinations for the two test organisms which we analyzed in this study. The first test battery was for gram-negative organisms in general, including urinary tract isolates. The second battery was for *Pseudomonas aeruginosa* strains. A third battery is routinely used in most clinical microbiology laboratories for gram-positive organisms, including enterococci, but was not evaluated in this study.

Antimicrobial agents were received directly from the manufacturer (Pfizer Diagnostics Division) and were held in a frozen state (-20°C) until they were to

TABLE 1. *Antimicrobial agents tested*

Test strain	Antimicrobial agent	Disk content (μ g)
<i>E. coli</i> ATCC 29194	Amikacin	10
	Ampicillin	4.5
	Carbenicillin	120
	Cephalothin	15
	Chloramphenicol	4
	Colistin	13
	Gentamicin	9
	Nitrofurantoin	15
	Tobramycin	10
<i>P. aeruginosa</i> ATCC 27853	Trimethoprim-sulfamethoxazole (1:19)	18
	Amikacin	10
	Carbenicillin	120
	Chloramphenicol	4
	Colistin	13
	Gentamicin	9
	Tobramycin	10

be used for susceptibility testing. At that time, each cartridge was removed from the freezer, allowed to equilibrate to room temperature in a desiccator jar, and then quality control tested before being used for regular clinical testing on the next day. All cartridges placed in the disk dispenser were kept in a refrigerator (4°C) when not being used. The amikacin disk became available late in the study and, therefore, was tested fewer times than other study compounds.

Testing cartridges before putting them into service is a routine which we have practiced for years with the Kirby-Bauer susceptibility testing method.

Control organisms. Two control organisms were used for this particular evaluation, *E. coli* ATCC 29194 and *P. aeruginosa* ATCC 27853. We did not test *Staphylococcus aureus* in this particular evaluation. The control organisms were held in blood agar base medium, sealed, and kept in the dark at room temperature for interim day-to-day subcultures. The master reference subcultures for these strains were maintained in a lyophilized state. A new, lyophilized *P. aeruginosa* isolate was subcultured each week for routine testing through that 7-day period. The *E. coli* strain, consistently reliable when held at room temperature in the dark, was subcultured from the lyophilized stock once per month.

Autobac procedure. Autobac susceptibility testing was carried out according to the manufacturer's directions. There were no deviations from this procedure throughout the course of the evaluation. Instrument quality control was carried out by a single technologist assigned that responsibility, and instrument performance parameters were measured daily. The "Autobac calibration wedge" was tested each morning to ensure appropriate calibration of the machine. Each time a new lot of Eugonic broth, saline, cuvettes, etc., was received, stringent quality control measures were used to assess the performance of these components of the susceptibility testing procedure.

Disk diffusion susceptibility testing. The disk diffusion susceptibility testing procedure was carried

out according to the guidelines originally described by Bauer et al. in 1966 (1), as modified by the Food and Drug Administration (3, 4) and more recently by the National Committee for Clinical Laboratory Standards (5). Disks were dispensed to the surface of the agar plate by extracting them from the cartridges used for Autobac susceptibility disk dispensing. They were placed on the surface of the Mueller-Hinton agar plates by means of flamed forceps. Mueller-Hinton agar plates (15 cm) were purchased as prepared media from BBL Microbiology Systems (Cockeysville, Md.). Our routine quality control procedure, which includes assessment of agar depth and agar pH, routine quality control organism performance, and sterility assessment, was used with the Mueller-Hinton agar plates.

Technologists. Six different technologists read the results of both Autobac and Kirby-Bauer disk diffusion zone size results over the assessment period. On any given day, a single technologist was assigned this duty, and the duty was rotated among the six technologists involved with the evaluation. Technologists had no access to prior information regarding zone sizes with the Kirby-Bauer procedure, and results were recorded on a blank result report sheet each day and later tabulated by one of the authors.

Interpretation. The interpretation of zone size results from the disk diffusion susceptibility testing carried out in this particular study was made strictly on the basis of zone sizes. No attempt was made to correlate the interpretability of susceptibility zone sizes with the result reported as a light-scattering index from the Autobac procedure. The intent of recording zone sizes was to determine day-to-day variation (reproducibility or precision) and to assess the feasibility of carrying out this disk diffusion procedure with Autobac susceptibility disks for the purpose of Autobac quality control. Zone sizes were measured with calipers, reading from the back of the susceptibility plate with a diffused light source.

RESULTS

Table 2 outlines the range of zone sizes, the number of determinations, the median zone size, and the number of determinations falling within a ± 3 -mm range of the mean.

Zone sizes were within a range of ± 3 mm for 99% of the tests performed with *E. coli* and within ± 3 mm for 98% of the tests performed with *P. aeruginosa*.

Results were consistently reproducible on a week-to-week basis, and there were no periods of time when results, if they were outside the ± 3 -mm range, seemed to be consistent in that regard. On 2 consecutive days the *E. coli* quality control strain, which had been subcultured from sheep blood agar plates to Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) tubes, demonstrated small colonies within the zone of inhibition of ampicillin. When this occurred on a second day, this particular control organism was resubcultured from the original lyophilized stock supply and the problem did

TABLE 2. Zone size results

Antimicrobial agent	Range of zone sizes recorded (mm)	No. of determinations	Mean zone size (mm)	No. of determinations within ± 3 mm of mean zone size
<i>E. coli</i> ATCC 29194				
Amikacin	18-20	23	20	23
Ampicillin	9-13	109	10	107
Carbenicillin	25-28	109	26	108
Cephalothin	13-16	109	14	109
Chloramphenicol	9-13	109	12	109
Colistin	12-15	109	13	109
Gentamicin	20-23	109	21	109
Nitrofurantoin	9-12	83	11	83
Tobramycin	19-22	109	20	108
Trimethoprim-sulfamethoxazole	22-24	109	23	109
<i>P. aeruginosa</i> ATCC 27853				
Amikacin	14-16	23	15	23
Carbenicillin	19-25	97	21	95
Colistin	11-15	97	12	97
Gentamicin	13-18	97	14	96
Tobramycin	16-22	97	19	94

not recur. No other problems or unusual performance results were encountered. All susceptibility disk cartridges were tested before being placed in use as regular susceptibility reagents. However, during the course of the study no cartridge was discarded because it did not meet original Autobac quality control standards.

DISCUSSION

In a study published in 1972, Blazevic et al. (2) reported on quality control testing with a disk antibiotic susceptibility test. In assessing the results of this particular study the authors indicated that most results for the Kirby-Bauer methodology were within ± 2 mm of the value established as the mean reference value. In that study, the authors also reported that there was greater reproducibility when disks were maintained in a frozen state versus being maintained at 4°C in a refrigerator. We have relied upon that study for our determination of the optimal means of holding disks before usage for clinical testing.

In this study we have arbitrarily determined that ± 3 mm from the mean disk zone value would be a sufficiently demanding expectation of a disk susceptibility quality control check for the Autobac instrument. In that sense, we are less rigid in our expectations than were the authors of the above report (2), but perhaps we are more realistic in terms of a more general anticipation of performance in many laboratories.

The overall reproducibility of the Autobac susceptibility testing method is impressive and relates well to the reproducibility reported under tight performance conditions by Blazevic et al.

(2). The quality control procedure is simple and relatively well standardized, as the disk diffusion susceptibility testing has been well studied and, if proposed methodology is followed, gives impressive reproducibility results. The quality control assessment we performed has reassured us as to disk potency and performance as an alternative to a system which provides values which are interpreted only at the high end of a numerical scale. The Autobac control strain of *E. coli* consistently exhibited a light-scattering index value of 1.00 for all antimicrobials tested. As indicated previously, this type of end-of-scale criterion may not detect aberrations of individual clinical isolate susceptibilities. The disk diffusion testing allows a semiquantitative, continuous-scale determination and is likely to detect even small test performance variations, unless the quality control strain is highly resistant. This was not the case with either of the strains used in this evaluation.

The results of this study validate the rigid controls for the manufacturing, distribution, and laboratory storage of the Autobac susceptibility disks. The manufacturer's suggestions for quality control appear to be adequate for a day-to-day determination of performance in the Autobac instrument, but we propose that the disk diffusion susceptibility testing procedure described here be used each time a new cartridge is to be used in the testing situation. This, we feel, would obviate the possibility of an aberration which might not be picked up by the "end-of-scale" quality control testing now routinely carried out with the Autobac instrument. It would also take into account the practicality of

day-to-day performance and, at the same time, provide the added security of a continuous-assessment quality control test. We further propose that the disks be kept frozen until a cartridge is assessed in the above manner and used for routine testing.

It is likely that a similar procedure would prove valuable for other automated procedures in which disks are the vehicles for antimicrobial delivery.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Peggy Ahlin, Supervisor, and the technologists of the Clinical Microbiology Laboratory at the University of Utah Medical Center, and the secretarial/editorial assistance of Constance Staples.

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